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14. ABSTRACT

This is the final report for the Predoctoral Taineeship Award (No. DAMD17-02-1-0567). The studies described in the original grant proposal have been completed. The major findings included the following: (i) We demonstrated, for the first time, that the naturally-occurring estrogen fatty acid esters have a differential, strong mitogenic effect in the fat-rich mammary tissues, and this effect was not observed with estradiol. (ii) We showed that the endogenous estrogen fatty acid esters are pathogenically more important than estradio for the selective induction of mammary tumor formation. (iii) We found that the estrogen esterase activity is higher in the breast tissue than in the uterus, which provides the bases for our hypothesis, the endogenous estrogen-fatty acid esters are mammary-selective estrogens for stimulation of cell growth and cancer formation.

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Introduction

This is the **final report** for the Predoctoral Traineeship Award (No. DAMD17-02-1-0567). I like to note that this Predoctoral Traineeship Award was originally awarded to Ms. Laura H. Mills in early 2002. Around June 2003, Ms. Laura Mills was approved for graduation with a PH.D. Degree. At the request of my supervisor (Professor Bao Ting Zhu), I was named as the replacement P.I. on this grant proposal shortly following Ms. Mills' departure from the project.

While serving as the replacement P.I., I helped to conduct additional studies required for the completion of this project and I have also helped with the analysis of some of the data originally obtained by Ms. Mills. Now the studies described in the original grant proposal by Ms. Laura Hook Mills have been completed.

Body

The Specicific Aims of the Original Proposal:

Aim 1: I will evaluate the stimulatory effects of E_2-17β-stearate and 4-hydroxyestradiol-17β-stearate, two representative estrogen-fatty acid esters, on the growth of mammary vs. uterine cells in ovariectomized female Sprague-Dawley rats. Their effects will be compared with the effects of unesterified E_2 or 4-hydroxyestradiol. The circulating levels of prolactin, FSH, and LH will also be determined in all animals, which will serve as an indicator of estrogen's action on the pituitary. During these animal experiments, I will also collect the pituitary, thymus, and liver, and if time allows, and the growth-stimulatory effects of esterified vs. unesterified estrogens in these organs will be determined and compared with their effects in the fat-rich mammary tissue.

Aim 2: I will evaluate the carcinogenic activity of E_2-17β-stearate and 4-hydroxyestradiol-17β-stearate on mammary vs. uterine cells in ovariectomized female Sprague-Dawley rats. The results will be compared and correlated with the growth stimulatory effects determined under Aim 1.

Aim 3: I will evaluate the activity of estrogen esterase (the enzyme that hydrolyzes estrogen-fatty acid esters to release bioactive estrogens) in the mammary tissue, and its activity will be compared with that found in other organs such as the uterus and liver. These analyses will provide insights into our understanding of the mechanisms underlying a preferential mitogenic action (and perhaps carcinogenic action) of estrogen-fatty acid esters in the breast over the uterus.

Major findings by Ms. Laura H. Mills (the original P.I.) and me (the replacement P.I.).

1. We have completed evaluating the stimulatory effects of E_2-17β-stearate on the growth of mammary vs. uterine cells in ovariectomized female Sprague-Dawley rats, and their effects were compared with the effects of unesterified E_2. Experimentally, 0.5 or 5 nmol of E_2-17β-stearate or E_2 was released daily to ovariectomized female rats through an Alzet pump implanted under the back skin of the animal for 10 or 23 days. The growth-stimulatory effect of E_2-17β-stearate and E_2 on mammary glandular cells was determined according to 5-BrdU indices, and their effect on the uterus was determined by measuring both the 5-BrdU-labeling index and the uterine wet weight. We found that chronic treatment of ovariectomized female rats with 0.5 or 5 nmol/day of E_2-17β-stearate for 10 or 23 days had a stronger stimulatory effect on mammary glandular cell proliferation than treatment with equimolar doses of E_2. In the uterus, however, E_2 was more active in stimulating the proliferation of uterine endometrial cells than E_2-17β-stearate at equimolar doses. Our results demonstrated, for the first time, that a naturally-occurring E_2-17β-fatty acid ester has a differential, strong mitogenic effect in the fat-rich mammary tissues, and this effect was not observed with E_2.

2. We have also determined the plasma levels of prolactin (PRL), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) in ovariectomized female rats treated with E_2-17β-stearate or E_2. We found that chronic administration of E_2 showed a stronger negative regulatory effect on the plasma LH levels than E_2-17β-stearate. The plasma levels of FSH in ovariectomized female rats were not significantly affected by treatment with E_2-17β-stearate or E_2. Chronic administration of E_2-17β-stearate or E_2 markedly increased the plasma PRL levels, but their effects were not significantly different from each other.

3. We have also evaluated the activity of the estrogen-fatty acid ester with E_2 for the induction of mammary vs. pituitary tumors in intact female ACI rats. Experimentally, the animals received an s.c. implantation of a 20 mg pellet (containing 18 μmol of an estrogen plus cholesterol or 100% cholesterol as the control) at 62 ± 3 days of their age, and the animals were monitored for a total period of 8 months. Among the 25 control animals, none developed mammary tumor and all lived healthily during the whole carcinogenesis experiment. Among the 26 animals receiving an E_2 pellet, all the animals developed a large pituitary tumor (average weight = 254 ± 83 mg), but only 8 of them developed mammary tumor(s). A majority of the animals died or had to be terminated early because of severe sickness, likely due to the presence of the large pituitary tumor. Among the 26 animals implanted with the same molar dose of an E_2-fatty acid ester preparation (containing 63% E_2-17β-stearate and 37% E_2-17β-palmitate), a total of 9 animals developed mammary tumor, and none of the animals died of non-breast cancer-related sickness. Only 3 animals (11.5%; all with mammary tumor) developed a pituitary tumor, with a slightly smaller average size (173 ± 75 mg). In conclusion, while E_2 was far stronger in stimulating the formation of pituitary tumor (100% incidence) than mammary tumor (30.7% incidence), E_2-fatty acid esters had a somewhat stronger activity than E_2 in inducing the mammary tumor but only had very weak activity in inducing pituitary tumor. Our data suggest that the endogenous E_2-fatty acid esters are pathogenically more important than E_2 for the *selective* induction of mammary tumor formation.

4. We have evaluated the activity of estrogen esterase (the enzyme that hydrolyzes estrogen-fatty acid esters to release bioactive estrogens) in the mammary tissue. We found that this esterase activity is higher in the breast tissue than in the uterus. This is a very interesting novel finding, which provided the basis for our hypothesis the endogenous estrogen-fatty acid esters are mammary-selective estrogens for the induction of cell growth and cancer formation.

Key Research Accomplishments

1. We demonstrated, for the first time, that a naturally-occurring estrogen fatty acid ester has a differential, strong mitogenic effect in the fat-rich mammary tissues, and this effect was not observed with E_2.

2. We showed that the endogenous estrogen fatty acid esters are pathogenically more important than E_2 for the *selective* induction of mammary tumor formation.

3. We found that the estrogen esterase activity is higher in the breast tissue than in the uterus, which provides the basis for our hypothesis the endogenous estrogen-fatty acid esters are mammary-selective estrogens for stimulating cell growth and cancer formation.

Reportable Outcomes

Listed below are papers and abstracts that have come out of this award, with the P.I.'s names highlighted.

Mills LH, Lee AJ and Parlow AF and Zhu BT [2001] Preferential growth stimulation of mammary glands over uterine endometrium in female rats by a naturally occurring estradiol-17β-fatty acid ester. Cancer Research 61: 5764-5770.

Lee AJ, **Mills LH**, Kosh JW, Conney AH and Zhu BT [2002] NADPH-dependent metabolism of estrone by human liver microsomes. Journal of Pharmacology and Experimental Therapeutics 300: 838-849.

Hook LL (maiden name for **Laura H. Mills**), Lee AJY and Zhu BT [2000] Differential stimulatory actions of estradiol-17β-stearate on the growth of rat mammary vs. uterine cells. Proceedings of the American Association for Cancer Research 41: 429, San Francisco.

Mills LH and Zhu BT [2001] Chronic administration of 17β-estradiol inhibits intramammary lymphocyte proliferation in female rats. Proceedings of the American Association for Cancer Research 42: 237, New Orleans, Louisiana.

Mills LH, Sowell JW, Chapman JM and Zhu BT [2002] Synthesis of 4-hydroxyestradiol-17β-stearate, an estrogen fatty acid ester, and its stable prodrug 4-hydroxyestradiol-3,4-diacetate 17β-stearate. The South Eastern Regional Meeting of the American Chemical Society (SERMACS), November 13-16, 2002, Charleston, South Carolina.

Mills LH, Lee WJ and Zhu BT [2003] Naturally-occurring estradiol-17β-fatty acid ester, but not estradiol-17β, preferentially induces the development of mammary tumor in female ACI rats. Proceedings of the American Association for Cancer Research 44: 835-836.

Mills LH, Lee WJ and Zhu BT [2003] Naturally-occurring estradiol-17β-fatty acid ester, but not estradiol-17β, preferentially induces the development of mammary tumor in female ACI rats. **Manuscript in preparation (to be submitted to Cancer Research).**

Listed below are some other papers published by the replacement P.I. during his graduate studies under the supervision of Dr. B. T. Zhu:

Liu ZJ, **Lee WJ** and Zhu BT [2005] Selective insensitivity of ZR-75-1 human breast cancer cells to 2-methoxyestradiol: Evidence for Type II 17β-hydroxysteroid dehydrogenase as the underlying cause. Cancer Research 65: 5802-5811.

Lee WJ and Zhu BT [2005] Modulation of DNA methylation by catechol-O-methyltransferase. Medical Hypotheses and Research 2: 325-337.

Lee WJ and Zhu BT [2005] Inhibition of enzymatic DNA methylation by caffeic acid and chlorigenic acid, two common catechol-containing coffee polyphenols. Carcinogenesis [August 4, 2005; Epub ahead of print] [in press].

Lee WJ, Shim JY and Zhu BT [2005] Mechanisms for inhibition of DNA methyltransferases by tea catechins and bioflavonoids. Molecular Pharmacology [July 21, 2005; Epub ahead of print] [in press].

Lee WJ and Zhu BT [2004] Strong inhibition of DNA methylation by caffeic acid and chlorigenic acid, two polyphenolic components present in coffee. Proceedings of the American Association for Cancer Research 45, Orlando, Florida.

Lee WJ and Zhu BT [2005] Modulation of DNA methylation by catechol-O-methyltransferase. Proceedings of the American Association for Cancer Research 46: abstract 2749. Anaheim, California. April 16-20, 2005.

Lee WJ and Zhu BT [2005] Modulation of the rate of enzymatic DNA methylation by catechol-O-methyltransferase. South Carolina Alliance for Cancer Chemoprevention, 3rd Annual Symposium, abstract 20, May 17-18, 2005.

Conclusions

The naturally-occurring estrogen fatty acid esters have a preferential mitogenic effect in the fat-rich mammary tissues compared to estradiol. Also, these endogenous estrogen fatty acid esters are pathogenically more important than estradiol for the induction of mammary tumor formation in the animal model.

References

Mills LH, Lee AJ and Parlow AF and Zhu BT [2001] Preferential growth stimulation of mammary glands over uterine endometrium in female rats by a naturally occurring estradiol-17β-fatty acid ester. Cancer Research 61: 5764-5770.

Lee AJ, **Mills LH**, Kosh JW, Conney AH and Zhu BT [2002] NADPH-dependent metabolism of estrone by human liver microsomes. Journal of Pharmacology and Experimental Therapeutics 300: 838-849.

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Appendices

Not included.